CUB-1 CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Not yet assigned
Group Art Unit	:	Not yet assigned
Applicants	:	Frederick B. Oleson et al.
Application No.	:	Not yet assigned
Filed	:	Concurrently herewith
For	:	METHODS FOR ADMINISTRATION OF ANTIBIOTICS

New York, New York
February 20, 2002

Hon. Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examining this application, please amend the application as follows:

IN THE SPECIFICATION

On page 1, immediately below the title, please delete the first paragraph and substitute therefor the following paragraph:

This application is a continuation of United States Patent Application No. 09/406,568, filed September 25, 1999, which in turn claims benefit of United

Express Mail No.
EL 889410434 US

States Provisional Application Nos. 60/101,828, filed September 25, 1998, and 60/125,750, filed March 24, 1999, all of which are herein incorporated by reference.

Please replace pages 5, 6, 9, 15, 16, 18 and 20 with replacement pages 5, 6, 9, 15, 16, 18 and 20 (enclosed at Appendix A).*

IN THE CLAIMS

Please cancel claims 16-25 and 27-33. Please replace claim 26 with amended claim 26:

26. (Amended) The method according to either of claims 1 or 6, wherein said administering is via oral, subcutaneous or intravenous administration.

REMARKS

The Specification

Applicants have amended the specification to add a cross-reference to the applications from which this application claims benefit.

Applicants have amended the specification to conform with corrections in the formal drawings submitted concurrently herewith. Specifically, applicants have amended the specification to recite Figures 1A, 1B, 2A and 2B as appropriate. See page 5, line 27; page 6, lines 3-5 and 23; page 9, lines 1-2; page 15, line 27; page 16, line 2; page 18, lines 19-21 and 28; and page 20, line 19.

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* Applicants have enclosed herewith a "Marked-up Version Showing Changes Made" at Appendix B, wherein deleted material is bracketed and added material is underlined.

The Claims

Applicants have canceled claims 16-25 and 27-33 to decrease filing fees. Applicants have amended claim 26 to delete reference to canceled claim 16. Applicants reserve the right to pursue the canceled subject matter in this application or in another application claiming benefit therefrom.

None of the amendments adds new matter. Their entry is requested.

Respectfully submitted,

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SUMMARY OF THE INVENTION

The present invention addresses the problem of skeletal muscle toxicity at high doses of lipopeptide antibiotics such as daptomycin, as well as quinupristin/dalfopristin. The invention provides methods for administering the antibiotic that minimizes skeletal muscle toxicity while simultaneously maintaining a sufficient efficacy level.

The process of the invention is characterized by administering less frequent doses comprising a higher concentration of an antibiotic. This protocol is both safer and more efficacious than administering more frequent doses of the antibiotic at lower concentrations. Thus, in one method of the invention, daptomycin is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity. In another method of the invention, a lipopeptide antibiotic other than daptomycin, such as a daptomycin derivative, A54145 or a derivative thereof, is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity. In a third method of the invention, quinupristin/dalfopristin is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity.

The methods of the invention are characterized by administering a high dose of an antibiotic that causes skeletal muscle toxicity at a dosage interval of 24 hours to once weekly. In one embodiment of the invention, daptomycin is administered at a dose of 3 to 75 mg/kg at a dosage interval of 24 hours to once weekly. In another embodiment of the invention, quinupristin/dalfopristin is administered at a dose of 7.5 to 75 mg/kg at a dosage interval of 24 hours to once weekly.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B. Serum creatine phosphokinase (CPK) levels for Dog Study A (Fig. 1A) and Dog Study B (Fig. 1B). Serum CPK levels were

determined at two hours after daptomycin dosing as an indication of muscle toxicity.

Figures 2A and 2B. Steady state plasma concentrations of daptomycin on day 18 of dosing as determined by HPLC for Dog Study A (Fig. 2A) and Dog Study B (Fig. 2B).

Figure 3. Relationship between different dosing intervals of daptomycin and its skeletal muscle toxicity (related to CPK levels) and its effectiveness (related to the peak serum concentration, C_{max}, over the minimal inhibitory concentration, MIC, of daptomycin).

DETAILED DESCRIPTION OF THE INVENTION

To investigate the potential effects of dose fractionation on toxicity, two studies were conducted in dogs comparing the effects of repeated intravenous administration once daily (q24h) versus every 8 hours (q8h). These studies were conducted in the dog since this species is most predictive of clinical effects. The objective of the studies was to assess the relationship between pharmacokinetics, including C_{max} and AUC_{24h}, and skeletal muscle toxicity, in order to determine the optimal clinical dosing regimen to minimize potential for skeletal muscle toxicity.

Study A explored whether daptomycin-related skeletal muscle toxicity is related to the peak concentration of daptomycin that occurs in the bloodstream after administration (C_{max}) and not to the total concentration of daptomycin in the bloodstream for 24 hours (AUC_{24h}). In Study A, the daptomycin daily dose was fractionated into multiple administrations per day to reduce C_{max} (see Example 1 and Figure 2A).

Study B examined whether a threshold plasma concentration exists for daptomycin-related skeletal muscle toxicity. Under this hypothesis, administration of the no observed effect dose level at 24 hours (NOELq24h) multiple times per day, such that plasma levels of daptomycin remain below some

daptomycin and the presence of regenerative changes in skeletal muscle (Figures 1A and 1B). In addition, because C_{max} and/or AUC are the key determinants of efficacy in animal models of infection, the pharmacological activity of daptomycin is optimized by once-daily dosing. Therefore, because safety and efficacy are not dependent upon the same determinant (C_{max}), the safety margin for daptomycin can be increased by altering the dosing regimen.

Based upon these results, the present invention provides methods for administering daptomycin that minimize skeletal muscle toxicity compared to prior methods for administering daptomycin. The methods may be used for human patients in clinical applications and in veterinary applications. The dose and dosage interval for the method is one that is safe and efficacious in clinical or veterinary applications. The method of the invention teaches, in general, that longer dosing intervals can provide for the administration of higher doses of daptomycin.

In one embodiment of the instant invention, the dose is 3 to 75 mg/kg daptomycin. In a preferred embodiment, the dose is 6 to 25 mg/kg. In a more preferred embodiment, the dose for humans patients is 6 to 12 mg/kg. Doses that may be used include 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22 or 25 mg/kg. In a preferred embodiment for veterinary applications, the dose is 3 to 25 mg/kg. Other doses higher than, intermediate to or less than these doses may also be used and may be determined by one skilled in the art following the methods of this invention.

In one embodiment of the instant invention, the dosage interval is 24 hours to once weekly. In a preferred embodiment, daptomycin is administered at a dosage interval of once every 24 hours, once every 48 hours, once every 72 hours, once every 96 hours, or once weekly. Administration at less frequent dosage intervals, such as once every 96 hours or once weekly, may be desirable for patients who have impaired renal function or who require hemodialysis. In a more preferred embodiment the dosage interval is 24 to 48 hours. In an even more preferred embodiment, the dosage interval is 24 hours. The preferred dosage interval for veterinary applications may be somewhat shorter or longer than the preferred

and related drugs, carbapenems, cephalosporins and related drugs, aminoglycosides, bacitracin, gramicidin, mupirocin, chloramphenicol, thiamphenicol, fusidate sodium, lincomycin, clindamycin, macrolides, novobiocin, polymyxins, rifamycins, spectinomycin, tetracyclines, vancomycin, teicoplanin, streptogramins, anti-folate agents including sulfonamides, trimethoprim and its combinations and pyrimethamine, synthetic antibacterials including nitrofurans, methenamine mandelate and methenamine hippurate, nitroimidazoles, quinolones, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, para-aminosalicylic acid (PAS), cycloserine, capreomycin, ethionamide, prothionamide, thiacetazone and viomycin. In a preferred embodiment, antibiotics that may be co-administered with daptomycin or other lipopeptide antibiotics according this invention include, without limitation, imipenen, amikacin, netilmicin, fosfomycin, gentamicin, ceftriaxone and teicoplanin.

EXAMPLE 1

<u>STUDY A:</u> <u>EFFECT OF C_{MAX} ON CPK AND SKELETAL MUSCLE TOXICITY</u>

In order to study the effects of C_{max} on skeletal muscle toxicity, dogs (4 male dogs/group) were administered dose regimens of saline q8h, daptomycin 25 mg/kg q24h, daptomycin 75 mg/kg q24h and daptomycin 25 mg/kg q8h intravenously for 20 days. Skeletal muscle toxicity was measured in dogs by increases in CPK levels to above the normal range and by microscopic changes in skeletal tissue.

Steady state plasma concentrations of daptomycin on day 18 of dosing were determined by HPLC. C_{max} levels were approximately the same (1.23-fold higher) at 25 mg/kg q8h compared to 25 mg/kg q24h. C_{max} levels were approximately 2.8-fold higher at 75 mg/kg q24h compared to 25 mg/kg q8h. See Figure 1A (Study A). The AUC was approximately the same (0.37-fold

higher) at 25 mg/kg q8h compared to 75 mg/kg q24h (see Table 2 and Figure 2A).

Throughout the treatment period in Study A, a dose-proportional increase in peak CPK activity was apparent when the dose was increased from 25 to 75 mg/kg at a constant q24h dosing interval. However, an additional 4-fold increase in CPK levels were observed in animals dosed at 25 mg/kg q8h as compared with those dosed at 75 mg/kg q24h, even though the total daily dose for these two regimens was the same. For all dose regimens, CPK peaked after approximately 1 week of treatment, then declined despite continued treatment.

Treated animals were sacrificed at approximately one dosing interval after the last dose and muscle tissue was microscopically examined for indications of myopathy. See Table 1.

TABLE 1

	Dose Regimen			
Site Lesion*	**Saline q8h**	**25 mg/kg q24h**	**75 mg/kg q24h**	**25 mg/kg q8h**
Skeletal muscle				
Myofiber degeneration	0/24	3/24	8/24	14/24
Myofiber regeneration	1/24	2/24	1/24	9/24
Diaphragm				
Myofiber degeneration	0/4	0/4	0/4	1/4
Heart				
Myofiber degeneration	0/4	0/4	0/4	0/4

* The incidence of muscle-related histopathological findings is presented as the number of sites affected divided by the number of sites examined. For skeletal muscle, six sites were examined in each of four dogs for a total of 24 sites.

Skeletal myofiber degeneration increased approximately two-fold at 25 mg/kg q8h compared to 75 mg/kg q75h. In addition, skeletal myofiber degeneration increase five-fold at 25 mg/kg q8h compared to 25 mg/kg q24h. The skeletal myofiber degeneration was of minimal severity, correlating to three- to 25-

concentrations, allowing for more time for repair and, therefore, less toxicity. For example, at a dose regiment of 25 mg/kg q8h, the plasma concentrations never fell below 27 μg/mL, the trough value for this regimen. In contrast, plasma concentrations for the 75 mg/kg q24h regimen were below this level for approximately 12 hours prior to administration of the next dose. This daily period of minimal exposure may explain why the once-daily dosing regimen (75 mg/kg q24h) was associated with less toxicity than fractionated dosing (25 mg/kg q8h).

EXAMPLE 2

STUDY B: EFFECT OF THRESHOLD PLASMA CONCENTRATION ON SKELETAL MUSCLE TOXICITY

In order to study the effects of threshold plasma concentration on skeletal muscle toxicity, dogs (4 male dogs/group) were administered dose regimens of saline q8h, daptomycin 5 mg/kg q24h (approximate NOELq24h) and daptomycin 5 mg/kg q8h intravenously for 20 days.

As in Example 1, steady state plasma concentrations of daptomycin on day 18 of dosing were determined by HPLC. The q8h interval represents 3 half-lives in dogs ($t_{1/2}$ = 2.5 hours) and should have minimal impact on steady state C_{max} as compared to a q24h regimen. The C_{max} for 5 mg/kg q8h and 5 mg/kg q24h was approximately the same for both dose regimens. See Figure 1B (Study B). However, the AUC was approximately three-fold higher (2.6-fold higher) at 5 mg/kg q8h compared to 5 mg/kg q24h (see Table 4 and Figure 2B).

Serum CPK levels were determined as disclosed in Example 1. There were no changes in CPK levels at 5 mg/kg q24h compared to the saline control. In contrast, CPK levels at 5 mg/kg q8h were elevated compared to 5 mg/kg q24h or saline controls. At 5 mg/kg q8h, CPK levels peaked at levels three- to four-fold higher than baseline after one week of daptomycin treatment, and declined thereafter despite continued treatment, similar to what was seen in Study A. See Figure 1B (Study B).

The findings of Study B are summarized in Table 4:

TABLE 4

Dose Regimen	Total Daily Dose (mg/kg)	C_{max} (μg/mL)	AUC_{0-24h} (μg-h/mL)	Peak CPK (U/L)	Incidence of Micro-scopic Myopathy[1]
saline q8h	0	0	0	150	0/28
5 mg/kg q24h	5	58	180	150	3/28
5 mg/kg q8h	15	58	412	500	11/28

[1] The incidence of microscopic myopathy (last column) shows the number of sites that exhibit minimal degenerative changes divided by the number of sites examined. In this experiment, seven sites were examined in each of four dogs for a total of 28 sites.

At a q24h dosing interval, the NOEL is approximately 5 mg/kg. This NOELq24h results in no CPK changes and only very minimal histopathological evidence of skeletal muscle toxicity. However, these experiments demonstrate that the NOELq24h does not define a threshold plasma concentration for toxicity because administration every 8 hours (i.e., 5 mg/kg q8h) leads to skeletal muscle toxicity evident by increases in CPK and microscopic myopathy even though the C_{max} was similar to that of the 5 mg/kg q24h regimen. Toxicity may be related to time below a given plasma concentration. For example, time below 10 μg/mL is 6 hours at 5 mg/kg q8h compared to 18 hours at 5 mg/kg q24h. See Figure 1B. These results suggest that the peak plasma concentration of daptomycin associated with no observable skeletal muscle toxicity is dependent upon dosing frequency.

EXAMPLE 3

In order to study the effects of C_{max} of quinupristin/dalfopristin on skeletal muscle toxicity, dogs (4 male dogs/group) are administered dose regimens

SUMMARY OF THE INVENTION

The present invention addresses the problem of skeletal muscle toxicity at high doses of lipopeptide antibiotics such as daptomycin, as well as quinupristin/dalfopristin. The invention provides methods for administering the antibiotic that minimizes skeletal muscle toxicity while simultaneously maintaining a sufficient efficacy level.

The process of the invention is characterized by administering less frequent doses comprising a higher concentration of an antibiotic. This protocol is both safer and more efficacious than administering more frequent doses of the antibiotic at lower concentrations. Thus, in one method of the invention, daptomycin is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity. In another method of the invention, a lipopeptide antibiotic other than daptomycin, such as a daptomycin derivative, A54145 or a derivative thereof, is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity. In a third method of the invention, quinupristin/dalfopristin is administered to a patient in need thereof at a dosing interval that minimizes skeletal muscle toxicity.

The methods of the invention are characterized by administering a high dose of an antibiotic that causes skeletal muscle toxicity at a dosage interval of 24 hours to once weekly. In one embodiment of the invention, daptomycin is administered at a dose of 3 to 75 mg/kg at a dosage interval of 24 hours to once weekly. In another embodiment of the invention, quinupristin/dalfopristin is administered at a dose of 7.5 to 75 mg/kg at a dosage interval of 24 hours to once weekly.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B [Figure 1]. Serum creatine phosphokinase (CPK) levels for Dog Study A (Fig. 1A [top panel]) and Dog Study B (Fig. 1B [bottom panel]). Serum CPK levels were

determined at two hours after daptomycin dosing as an indication of muscle toxicity.

Figures 2A and 2B [Figure 2]. Steady state plasma concentrations of daptomycin on day 18 of dosing as determined by HPLC for Dog Study A (Fig. 2A [top panel]) and Dog Study B (Fig. 2B [bottom panel]).

Figure 3. Relationship between different dosing intervals of daptomycin and its skeletal muscle toxicity (related to CPK levels) and its effectiveness (related to the peak serum concentration, C_{max}, over the minimal inhibitory concentration, MIC, of daptomycin).

DETAILED DESCRIPTION OF THE INVENTION

To investigate the potential effects of dose fractionation on toxicity, two studies were conducted in dogs comparing the effects of repeated intravenous administration once daily (q24h) versus every 8 hours (q8h). These studies were conducted in the dog since this species is most predictive of clinical effects. The objective of the studies was to assess the relationship between pharmacokinetics, including C_{max} and AUC_{24h}, and skeletal muscle toxicity, in order to determine the optimal clinical dosing regimen to minimize potential for skeletal muscle toxicity.

Study A explored whether daptomycin-related skeletal muscle toxicity is related to the peak concentration of daptomycin that occurs in the bloodstream after administration (C_{max}) and not to the total concentration of daptomycin in the bloodstream for 24 hours (AUC_{24h}). In Study A, the daptomycin daily dose was fractionated into multiple administrations per day to reduce C_{max} (see Example 1 and Figure 2A [2, top panel]).

Study B examined whether a threshold plasma concentration exists for daptomycin-related skeletal muscle toxicity. Under this hypothesis, administration of the no observed effect dose level at 24 hours (NOELq24h) multiple times per day, such that plasma levels of daptomycin remain below some

daptomycin and the presence of regenerative changes in skeletal muscle (Figures 1A and 1B [Figure 1]). In addition, because C_{max} and/or AUC are the key determinants of efficacy in animal models of infection, the pharmacological activity of daptomycin is optimized by once-daily dosing. Therefore, because safety and efficacy are not dependent upon the same determinant (C_{max}), the safety margin for daptomycin can be increased by altering the dosing regimen.

Based upon these results, the present invention provides methods for administering daptomycin that minimize skeletal muscle toxicity compared to prior methods for administering daptomycin. The methods may be used for human patients in clinical applications and in veterinary applications. The dose and dosage interval for the method is one that is safe and efficacious in clinical or veterinary applications. The method of the invention teaches, in general, that longer dosing intervals can provide for the administration of higher doses of daptomycin.

In one embodiment of the instant invention, the dose is 3 to 75 mg/kg daptomycin. In a preferred embodiment, the dose is 6 to 25 mg/kg. In a more preferred embodiment, the dose for humans patients is 6 to 12 mg/kg. Doses that may be used include 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22 or 25 mg/kg. In a preferred embodiment for veterinary applications, the dose is 3 to 25 mg/kg. Other doses higher than, intermediate to or less than these doses may also be used and may be determined by one skilled in the art following the methods of this invention.

In one embodiment of the instant invention, the dosage interval is 24 hours to once weekly. In a preferred embodiment, daptomycin is administered at a dosage interval of once every 24 hours, once every 48 hours, once every 72 hours, once every 96 hours, or once weekly. Administration at less frequent dosage intervals, such as once every 96 hours or once weekly, may be desirable for patients who have impaired renal function or who require hemodialysis. In a more preferred embodiment the dosage interval is 24 to 48 hours. In an even more preferred embodiment, the dosage interval is 24 hours. The preferred dosage interval for veterinary applications may be somewhat shorter or longer than the preferred

and related drugs, carbapenems, cephalosporins and related drugs, aminoglycosides, bacitracin, gramicidin, mupirocin, chloramphenicol, thiamphenicol, fusidate sodium, lincomycin, clindamycin, macrolides, novobiocin, polymyxins, rifamycins, spectinomycin, tetracyclines, vancomycin, teicoplanin, streptogramins, anti-folate agents including sulfonamides, trimethoprim and its combinations and pyrimethamine, synthetic antibacterials including nitrofurans, methenamine mandelate and methenamine hippurate, nitroimidazoles, quinolones, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, para-aminosalicylic acid (PAS), cycloserine, capreomycin, ethionamide, prothionamide, thiacetazone and viomycin. In a preferred embodiment, antibiotics that may be co-administered with daptomycin or other lipopeptide antibiotics according this invention include, without limitation, imipenen, amikacin, netilmicin, fosfomycin, gentamicin, ceftriaxone and teicoplanin.

EXAMPLE 1

STUDY A: EFFECT OF C_{MAX} ON CPK AND SKELETAL MUSCLE TOXICITY

In order to study the effects of C_{max} on skeletal muscle toxicity, dogs (4 male dogs/group) were administered dose regimens of saline q8h, daptomycin 25 mg/kg q24h, daptomycin 75 mg/kg q24h and daptomycin 25 mg/kg q8h intravenously for 20 days. Skeletal muscle toxicity was measured in dogs by increases in CPK levels to above the normal range and by microscopic changes in skeletal tissue.

Steady state plasma concentrations of daptomycin on day 18 of dosing were determined by HPLC. C_{max} levels were approximately the same (1.23-fold higher) at 25 mg/kg q8h compared to 25 mg/kg q24h. C_{max} levels were approximately 2.8-fold higher at 75 mg/kg q24h compared to 25 mg/kg q8h. See Figure 1A [top panel] (Study A). The AUC was approximately the same (0.37-fold

and related drugs, carbapenems, cephalosporins and related drugs, aminoglycosides, bacitracin, gramicidin, mupirocin, chloramphenicol, thiamphenicol, fusidate sodium, lincomycin, clindamycin, macrolides, novobiocin, polymyxins, rifamycins, spectinomycin, tetracyclines, vancomycin, teicoplanin, streptogramins, anti-folate agents including sulfonamides, trimethoprim and its combinations and pyrimethamine, synthetic antibacterials including nitrofurans, methenamine mandelate and methenamine hippurate, nitroimidazoles, quinolones, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, para-aminosalicylic acid (PAS), cycloserine, capreomycin, ethionamide, prothionamide, thiacetazone and viomycin. In a preferred embodiment, antibiotics that may be co-administered with daptomycin or other lipopeptide antibiotics according this invention include, without limitation, imipenen, amikacin, netilmicin, fosfomycin, gentamicin, ceftriaxone and teicoplanin.

EXAMPLE 1

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Steady state plasma concentrations of daptomycin on day 18 of dosing were determined by HPLC. C_{max} levels were approximately the same (1.23-fold higher) at 25 mg/kg q8h compared to 25 mg/kg q24h. C_{max} levels were approximately 2.8-fold higher at 75 mg/kg q24h compared to 25 mg/kg q8h. See Figure 1A [1, top panel] (Study A). The AUC was approximately the same (0.37-fold

concentrations, allowing for more time for repair and, therefore, less toxicity. For example, at a dose regiment of 25 mg/kg q8h, the plasma concentrations never fell below 27 μg/mL, the trough value for this regimen. In contrast, plasma concentrations for the 75 mg/kg q24h regimen were below this level for approximately 12 hours prior to administration of the next dose. This daily period of minimal exposure may explain why the once-daily dosing regimen (75 mg/kg q24h) was associated with less toxicity than fractionated dosing (25 mg/kg q8h).

EXAMPLE 2

STUDY B: EFFECT OF THRESHOLD PLASMA CONCENTRATION ON SKELETAL MUSCLE TOXICITY

In order to study the effects of threshold plasma concentration on skeletal muscle toxicity, dogs (4 male dogs/group) were administered dose regimens of saline q8h, daptomycin 5 mg/kg q24h (approximate NOELq24h) and daptomycin 5 mg/kg q8h intravenously for 20 days.

As in Example 1, steady state plasma concentrations of daptomycin on day 18 of dosing were determined by HPLC. The q8h interval represents 3 half-lives in dogs ($t_{1/2}$ = 2.5 hours) and should have minimal impact on steady state C_{max} as compared to a q24h regimen. The C_{max} for 5 mg/kg q8h and 5 mg/kg q24h was approximately the same for both dose regimens. See Figure 1B [1, bottom panel] (Study B). However, the AUC was approximately three-fold higher (2.6-fold higher) at 5 mg/kg q8h compared to 5 mg/kg q24h (see Table 4 and Figure 2B [2, bottom panel).

Serum CPK levels were determined as disclosed in Example 1. There were no changes in CPK levels at 5 mg/kg q24h compared to the saline control. In contrast, CPK levels at 5 mg/kg q8h were elevated compared to 5 mg/kg q24h or saline controls. At 5 mg/kg q8h, CPK levels peaked at levels three- to four-fold higher than baseline after one week of daptomycin treatment, and declined thereafter despite continued treatment, similar to what was seen in Study A. See Figure 1B [1, bottom panel] (Study B).

The findings of Study B are summarized in Table 4:

TABLE 4

Dose Regimen	Total Daily Dose (mg/kg)	C_{max} (μg/mL)	$AUC_{0\text{-}24h}$ (μg-h/mL)	Peak CPK (U/L)	Incidence of Microscopic Myopathy[1]
saline q8h	0	0	0	150	0/28
5 mg/kg q24h	5	58	180	150	3/28
5 mg/kg q8h	15	58	412	500	11/28

[1] The incidence of microscopic myopathy (last column) shows the number of sites that exhibit minimal degenerative changes divided by the number of sites examined. In this experiment, seven sites were examined in each of four dogs for a total of 28 sites.

At a q24h dosing interval, the NOEL is approximately 5 mg/kg. This NOELq24h results in no CPK changes and only very minimal histopathological evidence of skeletal muscle toxicity. However, these experiments demonstrate that the NOELq24h does not define a threshold plasma concentration for toxicity because administration every 8 hours (i.e., 5 mg/kg q8h) leads to skeletal muscle toxicity evident by increases in CPK and microscopic myopathy even though the C_{max} was similar to that of the 5 mg/kg q24h regimen. Toxicity may be related to time below a given plasma concentration. For example, time below 10 μg/mL is 6 hours at 5 mg/kg q8h compared to 18 hours at 5 mg/kg q24h. See Figure 1B [1, bottom panel].

These results suggest that the peak plasma concentration of daptomycin associated with no observable skeletal muscle toxicity is dependent upon dosing frequency.

EXAMPLE 3

In order to study the effects of C_{max} of quinupristin/dalfopristin on skeletal muscle toxicity, dogs (4 male dogs/group) are administered dose regimens

26. The method according to [any one] either of claims 1[,] or 6 [or 16], wherein said administering is via oral, subcutaneous or intravenous administration.